Summary
During hematopoiesis, the survival of progenitor cells is controlled by a network of cytokines and adhesion molecules that activate a number of signaling pathways aimed to inhibit apoptosis. Apoptosis, or programmed cell death, is a normal physiological process that eliminates unneeded, old, or damaged cells. Selected genes regulate a cascade of signaling pathways in the cell, causing targeted cells to commit suicide. This process is important to embryonic development, the daily maintenance of body systems, and the prevention of cellular overgrowth. Its disruption, however, contributes to many disorders ranging from cancer and autoimmune diseases to degenerative syndromes. Without this death signal, tumor cells can be allowed to grow uncontrolled. Apoptosis is currently the focus of intense research to better understand this process and to explain how it contributes to cancer development and how it controls tissue homeostasis.

Key proteins in the regulation of apoptosis
Hematopoietic progenitor cells require growth factors for survival, differentiation and proliferation. The absence of these growth factors leads to apoptosis, which is critical to control cell numbers within the hematopoietic compartment. In mammals, the executory arm of apoptosis involves a family of death proteases, called caspases, that are activated in a proteolytic cascade to execute the cell death program. The activation of upstream caspases represents a critical checkpoint in the decision to survive or to die. The Bcl-2 family of proteins play a central role in the regulation of caspases. Members of this family possess at least one of four conserved motifs known as Bcl-2 homology domains (BH1 to BH4), and can exert both pro-survival and pro-apoptotic activity. In addition, caspases can also be controlled downstream of Bcl-2 by the IAP (inhibitor of apoptosis) family of proteins, which appear to directly block caspase activity and/or activation.

Several growth factors, including interleukin-3 (IL-3) and erythropoietin (Epo), have been shown to maintain the expression of pro-survival Bcl-2 family members (i.e., Bcl-2, Bcl-xL, A1, and Mcl-1) at the transcriptional level. By contrast, proapoptotic members such as Bax, Bad, Bim and Bid, appear to undergo a post-translational regulation. Although IL-3 signaling regulates the transcription of the family members that function as cell death antagonists, only the down-regulation of Bcl-xL protein was consistent kinetically with a key role in regulating the apoptosis of myeloid progenitors. This is in agreement with the results found in bcl-x-deficient mice, in which the absence of this survival factor induced a lethal level of apoptosis within the hematopoietic system.

Survival pathways in hematopoietic progenitors
The intracellular molecular pathways that regulate the survival of primitive hematopoietic progenitors are still poorly understood. Models of erythropoiesis and granulopoiesis have been extensively used to demonstrate the need of growth factors to induce cell survival, proliferation and maturation and to show that apoptosis is a normal physiological process controlling homeostasis. Epo is the main factor involved in red cell production as the lack of Epo results in anemia, which can be efficiently treated with recombinant Epo. This erythroid growth and survival factor binds to a specific cell surface receptor that is expressed on erythroid progenitors. The Epo receptor associates with Jak2, a member of a subfamily of protein tyrosine kinases, which plays an important role in cytokine-dependent gene regulation. Activated Jak2, in turn, converts a latent cytoplasmic transcription factor, Stat5, into its active form by tyrosine phosphorylation. The activated Stat5 translocates into the nucleus, where it binds to specific DNA response elements in the promoter region of target genes and activates transcription. Stat5 belongs to a family of transcription factors that are activated in response to diverse cytokines and growth factors. It has been shown that Epo functions as a survival factor, at least in part by repressing apoptosis through Bcl-xL during proliferation and differentiation of erythroid progenitors. Consistent with this, an Epo-responsive motif for the binding of Stat5 has been identified in the untranslated 5' region of the mouse bcl-x gene. In Epo-dependent erythroid progenitors, this Stat5 motif is
active in response to Epo, a finding confirmed as site-directed mutagenesis abrogates its promoter activity and overexpression of a dominant negative Stat3 protein blocks transactivation of bel-x. These in vitro studies have been confirmed in Stat3-deficient mice. Stat3 knockout embryos are severely anemic and consistently, erythroid progenitors show higher levels of apoptosis and are less responsive to Epo. Stat5 has also been shown to induce the expression of Bcl-xL in hematopoietic progenitor cells in response to IL-3.

In a model of a human multiple myeloma cell line it has been shown that Stat3, another member of the Stat family, is constitutively activated as a result of aberrant upstream signals, and that this activation is induced by interleukin-6 and blocked by Jak inhibitors. Moreover, constitutive Stat3 up-regulates the expression of Bcl-xL, which is a crucial event for the survival of myeloma cells. All these data indicate that growth factor-dependent activation of Stats is a critical transcriptional pathway to induce the expression of Bcl-xL and consequently to inhibit apoptosis in hematopoietic cells.

Mediators of apoptosis in hematopoietic progenitors

As mentioned above, post-translational mechanisms control a number of pro-apoptotic members of the Bcl-2 family. Bid remains inert until cleaved by caspase 8, and Bim is retained on microtubules by the dynein light chain LC8, whereas phosphorylation of Bad by the serine/threonine kinase Akt allows its dissociation from the dynein light chain LC8 and consequently to inhibit apoptosis. Furthermore, DREAM inhibits the activity of another pro-apoptotic protein, Bad, through phosphorylation by Akt and inducing the expression of the anti-apoptotic Bcl-xL protein by a Jak-Stat transcriptional pathway (fig. 1).

Regulation of apoptosis in myeloproliferative syndromes

With the exception of chronic myelogenous leukemia (CML), all other myeloproliferative syndromes (polycythemia vera, essential thrombocythemia, and myelofibrosis) lack a molecular hallmark that define the pathogenesis of these clonal diseases. Numerous studies have been focussed on understanding the molecular basis of polycythemia vera (PV). Chromosomal abnormalities have long been known to be associated with PV, but structural chromosomal alterations are seen in about 15% of patients with PV at diagnosis, which indicates that the genetic changes responsible for the pathogenesis of PV are likely to be due to DNA mutations unable to be detected by cytogenetic methods. Although analysis of mutant mice has established an essential role for Epo and its receptor in the development of erythroid progenitors in vivo, several studies have failed to identify mutations in the Epo receptor gene that may render Epo-independent erythroid cells in PV.

That erythroid colonies from patients with PV can survive and undergo maturation in vitro in the absence of Epo suggests that the anti-apoptotic pathways usually activated via binding of Epo to its cognate receptor may be either constitutively active or induced by other growth factors. Since Epo induces the expression of Bcl-xL in erythroid progenitors, an attractive hypothesis is that the levels of Bcl-xL are maintained in PV erythroid cells regardless of the presence of Epo, which may allows these cells to survive in the absence of their physiologic stimulus. This hypothesis has been recently confirmed with the finding that the Epo-independent erythroid cells express high levels of Bcl-xL when cultured in the absence of Epo, and that the expression of Bcl-xL in bone marrow erythroid cells is significantly higher in patients with untreated PV than in those with other myeloproliferative disorders or secondary erythrocytosis. Since Stat5 transacti-
CML is another myeloproliferative syndrome characterized by the translocation t(9;22). This translocation produces a chimeric gene that encodes a 210 K Bcr-Abl oncoprotein with dysregulated tyrosine kinase activity. Expression of Bcr-Abl in hematopoietic cells induces inhibition of apoptosis, growth factor independence, alterations in cell-cell and cell-matrix interactions, and leukemogenesis. In addition, due to the anti-apoptotic activity of this oncogene, Bcr-Abl-expressing leukemic cells are highly resistant to chemotherapeutic drugs. Although it has been demonstrated that expression of Bcr-Abl is the initiating event in CML, the downstream signaling pathways involved in transformation are not fully understood. Nevertheless, some of the pathways activated by Bcr-Abl that prevent apoptosis in leukemic cells begin to be uncovered. The PI3K/Akt pathway may represent an important bridge between the survival signal triggered by Bcr-Abl and modulators of apoptosis, such as Bad. However, the capacity of Bcr-Abl to induce phosphorylation of Bad through Akt, does not account for the complete protection of CML cells from apoptosis, suggesting the existence of alternative Bad-independent survival pathways. Consistent with this, it has been shown that Bcr-Abl induces the expression of Bcl-xL through the constitutive activation of Stat5, and this seems to be a major survival pathway in CML cells as inhibition of Stat5 activation leads to down-regulation of Bcl-xL and loss of cell viability. Furthermore, when Bcr-Abl-expressing cell lines and CD34+ cells from CML patients are incubated with an inhibitor of the Bcr-Abl kinase activity such as STI571, Stat5 loses its ability to interact with the bcl-xL gene and consequently the expression of Bcl-xL is reduced and leukemic cells undergo apoptosis. This mechanistic understanding of Bcr-Abl signaling opens the possibility to develop novel therapies.
addressed to inhibit key molecules (i.e., Stat5, PI3K/Akt) that transduce survival signals in CML and may be other hematologic malignancies.

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References